

Phytochemical and Nutrient Composition of the Freeze-Dried Amazonian Palm Berry, *Euterpe oleraceae* Mart. (Acai)

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Euterpe oleraceae is a large palm tree indigenous to the Amazon River and its tributaries and estuaries in South America. Its fruit, known as acai, is of great economic value to native people. In this study, a standardized freeze-dried acai fruit pulp/skin powder was used for all analyses and tests. Among many findings, anthocyanins (ACNs), proanthocyanidins (PACs), and other flavonoids were found to be the major phytochemicals. Two ACNs, cyanidin 3-glucoside and cyanidin 3-rutinoside were found to be predominant ACNs; three others were also found as minor ACNs. The total content of ACNs was measured as 3.1919 mg/g dry weight (DW). Polymers were found to be the major PACs. The concentration of total PACs was calculated as 12.89 mg/g DW. Other flavonoids, namely, homoorientin, orientin, isovitexin, scoparin, and taxifolin deoxyhexose, along with several unknown flavonoids, were also detected. Resveratrol was found but at a very low concentration. In addition, components including fatty acids, amino acids, sterols, minerals, and other nutrients were analyzed and quantified. Total polyunsaturated fatty acid, total monounsaturated fatty acid, and total saturated fatty acids contributed to 11.1%, 60.2%, and 28.7% of total fatty acid. Oleic acid (53.9%) and palmitic acid (26.7%) were found to be the two dominant fatty acids. Nineteen amino acids were found; the total amino acid content was determined to be 7.59% of total weight. The total sterols accounted for 0.048% by weight of powder. The three sterols B-sitosterol, campesterol, and stigmasterol were identified. A complete nutrient analysis is also presented. Microbiological analysis was also performed.

KEYWORDS: *Euterpe oleraceae*; acai; anthocyanin; proanthocyanidin; flavonoid; resveratrol; nutrient; sterol; fatty acid; amino acid; microbiological test; shelf life

INTRODUCTION

Euterpe oleraceae Martius is a large palm tree indigenous to South America. It grows abundantly in the Amazon estuary and on floodplains, in swamps, and in upland regions. Also known as the Cabbage palm, *Euterpe oleraceae* bears a dark purple, berry-like fruit, clustered into bunches, that serves as a major food source for native and lower class people of Brazil,

Colombia, and Suriname (1). A juice prepared from the fruit, popularly called "acai" in Brazil, is consumed in a variety of beverages and food preparations. In addition to its economic value, different parts of *Euterpe oleraceae* were used as folk medicine by native people. For example, the fruit furnishes a dark green oil used in rural medicine, principally as an antidiarrheal agent (2). Recently, much attention has been paid to the antioxidant capacity of its fruit (also called acai) and its possible role as a "functional food" or food ingredient (3–5). However, the knowledge of its phytochemical and nutrient composition is still very limited, which put its health claims and possible role as a "functional food" in question.

Some anthocyanins (ACNs) and several other flavonoids have been reported in acai (6–9). Regarded as predominant phytochemicals in acai, ACNs were believed to be the major

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compounds that contributed to the overall antioxidant capacity (9). But the contribution of the anthocyanins to the overall antioxidant capacities of the fruit were estimated to be only approximately 10%, which suggested that compounds that have yet to be identified are the major antioxidants in acai (3). Except for dietary antioxidants, other components and nutrient composition are also very important when we try to evaluate the possible role of acai as a "functional food". However, our knowledge of these is still not complete. In this paper, another major objective was to provide information about other components, as well as report on a more complete profile of its nutrient composition than reported by others to date.

Last, microbiological and heavy metal analyses were performed in an attempt to provide additional information related to safety issues of freeze-dried acai.

MATERIALS AND METHODS

Plant Material. Freeze-dried acai (*Euterpe oleracea*) was obtained from K2A LLC (OptiAcai, Provo, Utah). Prior to freeze drying, the berries were obtained immediately within the harvesting areas in the Amazon delta estuaries within kilometers of the freeze-drying facility in Belem, Brazil. Within hours of harvesting, acai berries were frozen and stored at -20°C until transferred for freeze drying. The freeze-dried acai powder was kept at -20°C until analyzed.

Chemicals and Standards. *Phytochemical Analysis.* Standards of 3-*O*- β -glucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (six mixed anthocyanin standard, HPLC grade) were obtained from Polyphenols Laboratories (Sandnes, Norway). Resveratrol standard was purchased from Sigma (St. Louis, MO). Formic acid was purchased from Aldrich (St. Louis, MO). Methanol, acetonitrile, methylene chloride, and acetic acid (HPLC grade) were from Fisher (Fair Lawn, NJ). Sephadex LH-20 is product of Sigma (St. Louis, MO).

Quantitative Analysis of Sterols. Sterol standards were purchased from Sigma (St. Louis, MO). Hydrochloric acid, methanol, and other reagents were obtained from Fisher (Fair Lawn, NJ).

Fatty Acids, Amino Acids, and Nutrient Analysis. Standards of fatty acids were obtained from Nu-Check Prep (Elysian, MN). *O*-Phthaldehyde was from Anresco (San Francisco, CA); amino acid standard solution (2.5 $\mu\text{mol/mL}$), Brij 35 solution (30%, w/w), 2-mercaptoethanol (2-hydroxyethylmercaptan), L-norleucine, and ethylenediaminetetraacetic acid (EDTA) tetrasodium salt (hydrate) were from Sigma (St. Louis, MO). Potassium hydroxide (pellets), sodium hydroxide (pellets), hydrochloric acid (6 N volumetric solution), and boric acid were from Chempure (Bolivar, OH). Vitamins A, C, E, D, B1, B2, B3, B6, and B12, glucose, fructose, lactose, sucrose, maltose, folic acid, pantothenic acid, biotin, and inositol were from Sigma-Aldrich (St. Louis, MO). Elemental ion solutions were from Absolute Standards (Hamden, CT).

Microbiological Analysis. Lactose broth for aerobic organism culturing was from Sigma-Aldrich (St. Louis, MO); reinforced clostridial agar for anaerobic organism culturing and enumeration was from EM Science (Gibbstown, NJ). Petrifilm enumeration plates were from 3M Microbiology Products (St. Paul, MN).

Analysis of Anthocyanin and Other Flavonoids. Sample preparation of anthocyanin and other flavonoid analysis followed the method reported previously (10).

Chromatographic analyses were performed on an HP 1100 series HPLC (Hewlett-Packard, Palo Alto, CA) equipped with an autosampler/injector and diode array detector (DAD). A 4.6 mm \times 250 mm, 5.0 μm , Zorbax Stablebond analytical SB-C₁₈ column (Agilent Technologies, Rising Sun, MD) was used for separation. Elution was performed with mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol) using the gradient protocol previously described (10). Low-resolution electrospray mass spectrometry was performed with an Esquire 3000 ion trap mass spectrometer (MS) (Bruker Daltonics, Billerica, MA). The experimental conditions were the same as previously described (10). Anthocyanin identification was determined following previous research (11). Quantification of anthocyanin followed the procedure reported before (10). For other flavonoid analysis,

the experimental conditions were kept the same except that the ionization was changed from positive mode to negative mode.

Proanthocyanidin Analysis. Freeze-dried acai powder (5 g) was extracted with solvent containing acetone, water, and acetic acid (70:29.5:0.5, v/v, AWA). This solution was further fractioned by Sephadex LH-20 for proanthocyanidin analysis following the published method (12) for proanthocyanidin analysis.

Chromatographic analyses were performed on an HP 1100 series HPLC (Hewlett-Packard, Palo Alto, CA) equipped with an autosampler/injector, DAD, fluorescence detector (FLD), which was also coupled with an LCQ ion trap mass spectrometer equipped with an API chamber, and an ESI source. Normal phase separation of proanthocyanidins was performed on a 3.0 mm \times 150 mm, 5.0 μm , Luna Silica column (Phenomenex, Torrance, CA). Elution was performed using mobile phase A (dichloromethane/methanol/water/acetic acid; 82:14:2:2, v/v) and mobile phase B (methanol/water/acetic acid; 96:2:2, v/v). The flow rate was 0.8 mL/min, and detection was set using FLD with excitation at 276 nm and emission at 316 nm. Gradient is described as follows: 0–17.6% B, 0–30 min; 17.6–30.7% B, 30–45 min; 30.7–87.8% B, 45–50 min. The proanthocyanidins were confirmed by their chromatographic patterns and the molecular weights obtained by FLD and MS detector, respectively.

Resveratrol Analysis. A freeze-dried acai sample (1 g) was extracted with 20 mL of methanol. After the extract was centrifuged at 14 000 rpm at 4°C for 5 min, the supernatant was used for resveratrol analysis. The analysis was carried out in a HP 1100 HPLC equipped with diode array detector and a Phenomenex Luna phenyl-hexyl column (250 mm \times 4.6 mm) with prefilter. Elution was performed using mobile phase A (water/acetonitrile/acetic acid; 89:9:2, v/v) and mobile phase B (acetonitrile/water; 80:20, v/v). The flow rate was 1.0 mL/min, and detection was set up at 280 nm using the DAD. The gradient is described as follows: 0% B, 0–10 min; 0–40% B, 10–25 min; 40–100% B, 25–32 min; 100% B, 32–35 min.

Sterol Analysis. Quantitative analysis of sterols in acai freeze-dried powder was carried out in a Varian 3400cx gas chromatograph with a DB-5ms column (Varian, Palo Alto, CA) based on INA sterol method 109.001 (13).

Fatty Acids, Amino Acids, and Nutrient Analysis. Fat was determined by the AOAC method (AOAC 933.05) (14). Fatty acids were analyzed based on the AOAC method (AOAC 969.33) (14). Analysis was carried out in an HP 5890 series 2 GC (Hewlett-Packard, Palo Alto, CA) with a Supelco ST-2560 column (Supelco, Inc., Bellefonte, PA). Cholesterol was tested in a HP 5890 series 2 GC (Hewlett-Packard, Palo Alto, CA) using an AOAC method (AOAC 994.10) (14).

Protein was determined based on an AOAC method (AOAC 991.20) (14), and the measurement was conducted in a Kjeltc 2400 autosampler unit (Rose Scientific Ltd., Edmonton, Alberta, CA). Amino acids were obtained by hydrolysis from protein by 6 N HCl and then analyzed by ion-exchange chromatography. *O*-Phthaldehyde is used for postcolumn derivation. Analysis was carried out in Waters Alliance 2690 HPLC equipped with Waters fluorescence detector 474 (Waters Corporation, Milford, MA). A Hitachi L-7100 pump (Hitachi High Technologies America, San Jose, CA) was used for postcolumn derivation. An interaction AA511 cation-exchange column (Pierce Biotechnology, Rockford, IL) with guard column was used to separate amino acids. Elution was performed using mobile phase, and the detection was set at excitation 358 nm and emission 425 nm.

Analysis of minerals was performed in a Perkin-Elmer ICP Optima 4300 DV ICP-OES system (Perkin-Elmer Life And Analytical Sciences Inc., Wellesley, MA) according to the AOAC method (AOAC 984.27) (14). Measurements of vitamin C (AOAC 967.22) (14), sugars (AOAC 980.13) (14), moisture (AOAC 926.08) (14), and ash (AOAC 945.46) (14) were all based on AOAC methods. Heavy metal ion analysis was performed by an Agilent HP-7500a ICP-MS (Agilent Technologies, Palo Alto, CA) on a 5% HNO₃ digested solution of elemental species (1000 mg/100 mL). Analysis of retinol was based on a published method (15).

Microbiological and Heavy Metal Analysis. Employing aseptic techniques, we placed samples of freeze-dried acai in a sterile glass homogenizer tube with 5 mL of sterile water. Using a sterile

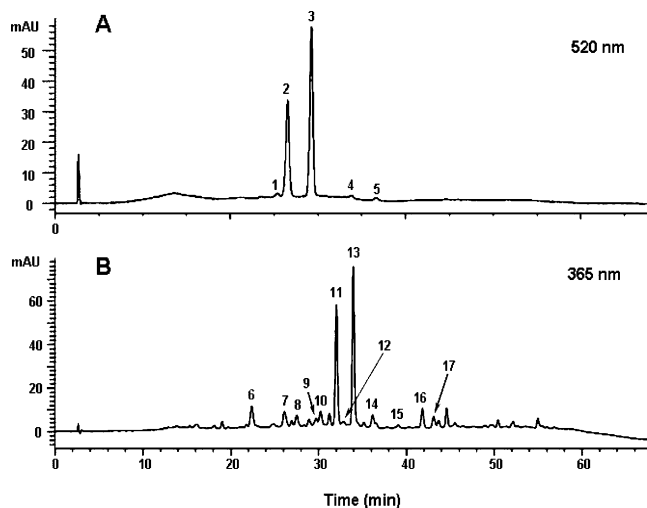


Figure 1. Reverse phase HPLC chromatograms of freeze-dried acai detected at 520 nm (A) and 360 nm (B). Peak identification and their MS data are shown in Table 1.

homogenizer beater, we crushed the berries to a pulp. Aliquots (1 mL) of the crushed berry pulp were placed into sterile lactose broth solutions and sterile reinforced clostridial agar solutions. The inoculated broth and agar solutions were incubated for 24 h at 37 °C under aerobic and anaerobic conditions, respectively. Following incubation, samples (1 mL) were plated onto growth-selective Petrifilm plates and spread in accordance with the supplier's instructions. Plates were subjected to incubation in accordance with the supplier's instruction. Enumeration was performed under a low powered (3×) light microscope with a hand held colony counter.

RESULTS AND DISCUSSION

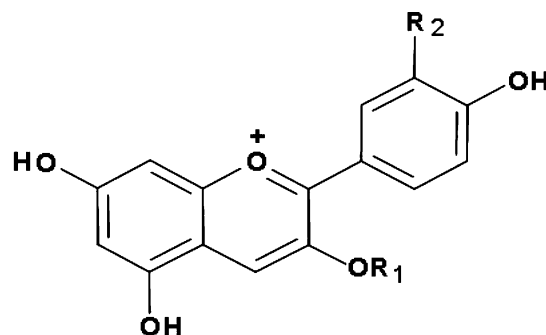
Identification and Quantification of Anthocyanins and Other Flavonoids. Five ACNs were identified from freeze-dried acai (Figure 1A). Of them, cyanidin 3-glucoside and cyanidin 3-rutinoside were found to be the predominant ACNs. Three minor ACNs, cyanidin 3-sambubioside, peonidin 3-glucoside, and peonidin 3-rutinoside, were also identified from acai. Among these minor ACNs, cyanidin 3-sambubioside and peonidin 3-glucoside were identified from acai for the first time. The MS spectral data and content of individual ACNs are presented in Table 1.

Like other berries rich in ACNs showing high antioxidant capacity (16), ACNs were believed to be the major antioxidant in freeze-dried acai. Nevertheless, freeze-dried acai was found to contain two major anthocyanins and their contents are much lower compared with that in most other berries (17). In this study, cyanidin 3-glucoside and cyanidin 3-rutinoside were found to be the major ACNs in freeze-dried acai, which agree with three previous reports (3, 6, 8). Three minor ACNs, namely, cyanidin 3-sambubioside, peonidin 3-glucoside, and peonidin 3-rutinoside, were detected in freeze-dried acai. But our results were not in accordance with two recent papers. In one of them (7), cyanidin 3-arabinoside and cyanidin 3-arabinosylarabinoside were identified as predominant ACNs, whereas in the other paper (9), only cyanidin 3-glucoside was found to be the predominant ACN, and pelargonidin 3-glucoside was identified as a minor ACN. Considering the significant difference of these ACN profiles of the plant materials, the results reported by these two papers (7, 9) have to be questioned. They were probably fruits either from other palm trees or other palm fruit varieties, rather than *Euterpe oleraceae* Mart. Thus, it is possible that in future study of acai, the ACN profile may be used as an alternative way to determine the plant materials. The total ACN

Table 1. Identification and Concentration of Anthocyanins and Other Flavonoids in Freeze-Dried Acai

peak no.	<i>t_R</i> (min)	MS (<i>m/z</i>)	MS/MS (<i>m/z</i>)	compounds	content (mg/g DW ^a)
Anthocyanins					
1	25.6	581	287	cyanidin 3-sambubioside	0.04
2	26.7	449	287	cyanidin 3-glucoside	1.17
3	29.4	595	449/287	cyanidin 3-rutinoside	1.93
4	33.9	^c	^c	peonidin 3-glucoside	0.02
5	36.6	609	463/301	peonidin 3-rutinoside	0.04
			total		3.19
Other Flavonoids					
6	22.4	689	671/609/489/369	unknown	^b
7	26.1	673	655/593/503/353	unknown	^b
8	27.5	391	289/221/143	unknown	^b
9	29.7	413	369/311/125	unknown	^b
10	30.2	449	327/269/151	unknown	^b
11	32.0	447	393/357/327	homoorientin	^b
12	32.4	373	341	unknown	^b
13	33.9	447	429/357/327/285	orientin	^b
14	36.2	431	341/311/283	unknown	^b
15	39.1	449	269/151	taxifolin deoxyhexose	^b
16	41.8	431	341/311/283	isovitexin	^b
17	43.1	461	407/371/341/309/231	scoparin	^b

^a Dry weight. ^b Not available. ^c Not determined.



Anthocyanins	R1	R2
Cyanidin-3-sambubioside	sambubioside	OH
Cyanidin-3-glucoside	glucose	OH
Cyanidin-3-rutinoside	rutinose	OH
Peonidin-3-glucoside	glucose	OMe
Peonidin-3-rutinoside	rutinose	OMe

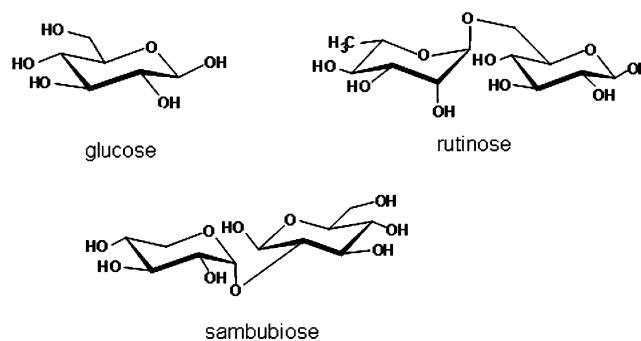


Figure 2. Chemical structures of anthocyanins in freeze-dried acai.

content in freeze-dried acai was 3.19 mg/g DW. It turned out to be lower than most other dark colored berries such as blueberries, blackberries, or cranberries (17).

Chemical structures of these ACNs are presented in Figure 2. Twelve other flavonoid-like compounds were also detected in acai (Figure 1B); five of them were identified as homoorientin, orientin, taxifolin deoxyhexose, isovitexin, and scoparin

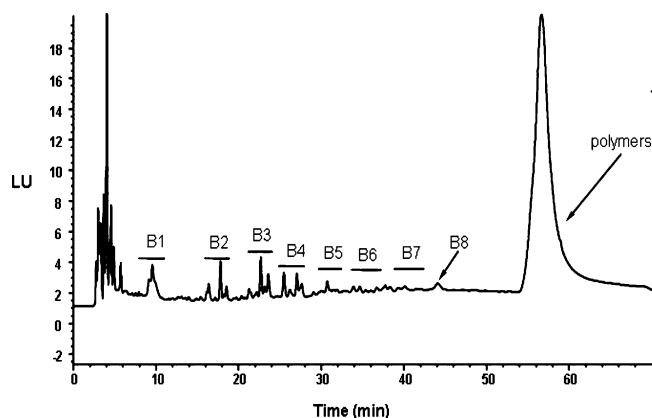


Figure 3. Normal phase HPLC chromatograms of freeze-dried acai detected by FLD with excitation at 276 nm and emission at 316 nm.

Table 2. Content of Proanthocyanidins in Freeze-Dried Acai

proanthocyanidins	content (mg/g, DW ^a)
monomers	0.21
dimers	0.30
trimers	0.25
tetramers	0.32
pentamers	0.31
hexamers	0.52
heptamers	0.32
octamers	0.39
nonamers	0.64
decamers	0.34
polymers	9.28
total	12.89

^a Dry weight.

by comparing their MS data with that from a published paper (8) (**Table 2**). However, quantification of these compounds failed due to lack of standards.

Characterization and Quantification of Proanthocyanidins. Proanthocyanidins (PACs) were found in acai as another group of polyphenolic compounds (3). In this study, proanthocyanidins were completely characterized and quantified in freeze-dried acai for the first time. Freeze-dried acai was found to contain monomers (epicatechin and catechin) and B type procyanidins from dimers to polymers, and polymers were found to be the major PACs in freeze-dried acai (**Figure 3**). The content of each group of proanthocyanidins is summarized in **Table 2**. Significantly, the profile of proanthocyanidins in freeze-dried acai is very similar to that of the blueberry (12). Proanthocyanidins have been found in most berries and have been found to possess strong antioxidant capacity, so they may contribute, at least partly, to overall *in vitro* antioxidant capacity. But being molecular compounds, in what forms they are absorbed or metabolized remains largely unknown. Therefore, their *in vivo* capacity as dietary antioxidants is still open to question.

Identification of Resveratrol. Resveratrol has been found primarily in grape skin and reported to exhibit chemopreventive properties against cancer (18, 19). Freeze-dried acai contained *trans*-resveratrol. However, the concentration is only 1.1 $\mu\text{g/g}$, which is probably too low to show actual chemopreventive effects, although this too remains an open question.

Fatty Acids, Amino Acids, Sterols, and Nutrient Analysis. Fatty acids, amino acids, sterols, heavy metal analysis, and a complete nutrient analysis of freeze-dried acai are reported in **Tables 3–6**, respectively. When the health benefits of a food

Table 3. Fatty Acids in Freeze-Dried Acai

fatty acids	formula	content (%)
Saturated Fatty Acids		
butyric	4:0	<0.1
caproic	6:0	<0.1
caprylic	8:0	<0.1
capric	10:0	<0.1
undecanoic	11:0	<0.1
lauric	12:0	0.1
tridecanoic	13:0	<0.1
myristic	14:0	0.2
pentadecanoic	15:0	<0.1
palmitic	16:0	24.1
margaric	17:0	0.1
stearic	18:0	1.6
nonadecanoic	19:0	<0.1
eicosanoic	20:0	<0.1
behenic	22:0	<0.1
tricosanoic	23:0	<0.1
lignoceric	24:0	<0.1
total		26.1
Monounsaturated Fatty Acids		
tridecenoic	13:1	<0.1
myristoleic	14:1	<0.1
pentadecenoic	15:1	<0.1
palmitoleic	16:1	4.3
margaroleic	17:1	0.1
oleic	18:1C	56.2
elaidic	18:1T	<0.1
gadoleic	20:1	<0.1
erucic	22:1	<0.1
nervonic	24:1	<0.1
total		60.6
Polyunsaturated Fatty Acids		
linoleic	18:2	12.5
linolenic	18:3	0.8
gamma linolenic	18:3G	<0.1
eicosadienoic	20:2	<0.1
eicosatrienoic	20:3	<0.1
homogamma linolenic	20:3G	<0.1
arachidonic	20:4	<0.1
eicosapentaenoic	20:5	<0.1
docosadienoic	22:2	<0.1
docosahexaenoic	22:6	<0.1
total		13.3

Table 4. Analysis of Amino Acids from Freeze-Dried Acai.

amino acids	result (%)
aspartic acid	0.83
threonine	0.31
serine	0.32
glutamic acid	0.80
glycine	0.39
alanine	0.46
valine	0.51
methionine	0.12
isoleucine	0.38
leucine	0.65
tyrosine	0.29
phenylalanine	0.43
lysine	0.66
histidine	0.17
arginine	0.42
proline	0.53
hydroxyproline	<0.01
cystine	0.18
tryptophan	0.13
total	7.59

are evaluated, the composition of other components and nutrients except for phytochemicals are analyzed. Nutrients

Table 5. Sterols in Freeze-Dried Acai

sterols	concentration (mg/g DW ^a)
β -sitosterol	0.44
campesterol	<0.03
stigmasterol	0.04
total	0.48

^a Dry weight.**Table 6.** Nutrient Analysis of Freeze-Dried Acai

analytes	result	unit per 100 g DW ^a
Label Analytes		
calories	533.9	
calories from fat	292.6	
total fat	32.5	g
saturated fat	8.1	g
cholesterol	13.5	Mg
sodium	30.4	Mg
total carbohydrate	52.2	g
dietary fiber	44.2	g
sugars	1.3	g
protein ($F = 6.25$)	8.1	g
vitamin A	1002	IU
vitamin C	<0.1	Mg
calcium	260.0	Mg
iron	4.4	Mg
Contributing Analytes		
moisture	3.4	g
ash	3.8	g
beta carotene	<5.0	IU
retinol	1002	IU
Sugar Profile		
fructose	0.4	g
lactose	<0.1	g
sucrose	<0.1	g
glucose	0.8	g
maltose	0.1	g

^a Dry weight.

preserved in freeze-dried acai may exert certain health effects. For instance, plant sterols have been found to have certain anticancer properties (20). A recent paper indicates that proteins in acai have inhibitory activity towards salivary α -amylase (21). In this paper, we present the complete analysis of fatty acids, sterols, amino acids, and other nutrients. Analysis of fatty acid composition revealed that the predominant fatty acid was oleic acid (56.2%), followed by palmitic acid (24.1%) and linoleic acid (12.5%). Total unsaturated fatty acid is 73.9% of all fatty acids. This result is largely in accordance with a previous report (22), though a more complete fatty acid composition was provided in this study. Five sterols were also found in acai in the paper mentioned above (22). However, β -sitosterol, campesterol, and stigmasterol were found in our study. Nineteen amino acids were found in freeze-dried acai for the first time. The total amino acid content is 7.59% of total weight. The nutrient composition of acai has previously been summarized in a book written in Portuguese based on several early studies (23). Unfortunately, data from different studies have not always agreed with each other. Besides, all the studies being summarized were conducted many years ago; two of them in the 1940s. Thus, we felt it might be useful to reanalyze the nutrient composition of acai. By adopting mostly AOAC procedures and new instrumentation, we tried to provide more accurate data compared with older data.

Table 7. Microbiological and Heavy Metal Analysis of Freeze-Dried Acai

analyte	result	unit
<i>Escherichia coli</i> /coliform (AOAC 991.14)	< 1	cfu/g
<i>Salmonella</i> (AOAC 989.13)	- ve	+/-
<i>Staphylococcus aureus</i> (AOAC 2000.15)	< 1	cfu/g
yeast and mold (AOAC 997.02)	^a	cfu/g
total aerobic (AOAC 990.12)	51600	cfu/g
Heavy Metals		
lead	36.77	ppb
arsenic	9.51	ppb
cadmium	9.41	ppb
mercury	1.58	ppb

^a Too numerous to count.

Microbiological and Heavy Metal Analysis. Microbiological and heavy metal analysis of freeze-dried acai is presented in **Table 7**. This information is highly related to safety and stability issues, and we hope this will be of help to those who are interested in developing products from acai.

Conclusion. The phytochemical and nutrient composition of *Euterpe oleraceae* Mart. has been investigated in this study. Anthocyanins (ACNs), proanthocyanidins (PACs), and other flavonoids were found to be the major phytochemicals in freeze-dried acai. The two most predominant ACNs found were cyanidin 3-glucoside and cyaniding 3-rutinoside, although their concentration was found to be lower than expected. For the first time, PACs were quantified and characterized in freeze-dried acai, with the majority of them being polymers. A complete analysis of fatty acids, sterols, amino acids, and other nutrients was also provided. The data obtained in the present study is crucially significant in advancing our understanding of the chemistry and therapeutic value of the Amazonian palm berry, *Euterpe oleraceae* Mart. (acai).

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